PB81-166605

Comparative Toxicity of Arsenic Compounds and Their Accumulation in Invertebrates and Fish

(U.S.) Environmental Research Lab.
Duluth, MN

1980

U.S. Department of Commerce
National Technical Information Service
NTIS

TECHNICAL REPORT DATA
(Please read Instructions on the reverse before completir

1. REPORT NO.	2.	3. PB81-166605
EPA-600/J-80-218	Journal Article	

4. TITLE AND SUBTITLE	5. REPORT DATE
Comparative Toxicity of Arsenic Compounds and Their Accumulation in Invertebrates and Fish	6. PERFORMING ORGANIZATION CODE

7. AUTHOR(S)	8. PERFORMING ORGANIZATION REPORT NO.
Robert L. Spehar, James T. Fiandt, Richard L. Anderson, and David L. DeFoe	

9. PERFORMING ORGANIZATION NAME AND ADDRESS	10. PROGRAM ELEMENT NO.
U.S. Environmental Protection Agency Environmental Research Laboratory-Duluth 6201 Congdon Boulevard Duluth, Minnesota 55804	11. CONTRACT/GRANT NO.

12. SPONSORING AGENCY NAME AND ADDRESS	13. TYPE OF REPORT AND PERIOD COVERED
Same as above	14. SPONSORING AGENCY CODE EPA-600/03

15. SUPPLEMENTARY NOTES

Arch. Environ. Contam. Toxicol. 9, 53-63 (1980).

16. ABSTRACT

The toxicity of arsenic III, arsenic V, sodium dimethyl arsenate, and disodium methyl arsenate to stoneflies, snails, amphipods, and trout, and the bioaccumulation of these compounds were studied during a 28-day flow-through test. Daphnia magna were exposed for 21 days in static tests to determine life-cycle effects. All animals were exposed to concentrations of approximately 100 to 1000 µg/L (as arsenic) of each of the compounds. Arsenic III, the most toxic compound, caused a significant reduction in the survival of amphipods at 1000 µg As/L after seven days. None of the compounds significantly affected the survival of other test species after 28 days or reduced young production in Daphnia after 14 days of exposure. The concentration of accumulated arsenic in stoneflies, snails, and Daphnia was as much as 131, 99, and 219 times greater than the water concentration, whereas amphipods and rainbow trout contained arsenic residues similar to the controls. Residues in stoneflies, snails, and Daphnia exposed to 1000 µg As/L were higher than those in animals exposed to 100 µg As/L, but appeared to reach a steady state after 14 days. Total arsenic accumulation was greatest in organisms exposed to inorganic arsenic, particularly at 100 µg/L.

17. KEY WORDS AND DOCUMENT ANALYSIS

a. DESCRIPTORS	b. IDENTIFIERS/OPEN ENDED TERMS	c. COSATI Field/Group

REPRODUCED BY
NATIONAL TECHNICAL
INFORMATION SERVICE
U.S. DEPARTMENT OF COMMERCE
SPRINGFIELD, VA 22161

18. DISTRIBUTION STATEMENT	19. SECURITY CLASS *(This Report)*	21. NO. OF PAGES
RELEASE TO PUBLIC	UNCLASSIFIED	11
	20. SECURITY CLASS *(This page)* UNCLASSIFIED	22. PRICE

EPA-600/J-80-218
Journal Article

Arch. Environm. Contam. Toxicol. 9, 53–63 (1980)

Archives of Environmental Contamination and Toxicology

Comparative Toxicity of Arsenic Compounds and Their Accumulation in Invertebrates and Fish

Robert L. Spehar, James T. Fiandt, Richard L. Anderson, and David L. DeFoe

U.S. Environmental Protection Agency, Environmental Research Laboratory-Duluth, 6201 Congdon Boulevard, Duluth, Minnesota 55804

Abstract. The toxicity of arsenic III, arsenic V, sodium dimethyl arsenate, and disodium methyl arsenate to stoneflies, snails, amphipods, and trout, and the bioaccumulation of these compounds were studied during a 28-day flow-through test. *Daphnia magna* were exposed for 21 days in static tests to determine life-cycle effects. All animals were exposed to concentrations of approximately 100 and 1000 μg/L (as arsenic) of each of the compounds. Arsenic III, the most toxic compound, caused a significant reduction in the survival of amphipods at 1000 μg As/L after seven days. None of the compounds significantly affected the survival of other test species after 28 days or reduced young production in *Daphnia* after 14 days of exposure. The concentration of accumulated arsenic in stoneflies, snails, and *Daphnia* was as much as 131, 99, and 219 times greater than the water concentration, whereas amphipods and rainbow trout contained arsenic residues similar to the controls. Residues in stoneflies, snails, and *Daphnia* exposed to 1000 μg As/L were higher than those in animals exposed to 100 μg As/L, but appeared to reach a steady state after 14 days. Total arsenic accumulation was greatest in organisms exposed to inorganic arsenic, particularly at 100 μg/L.

Arsenic compounds are found in all living organisms, including those in aquatic systems. Water concentrations in U.S. lakes and rivers have been reported to range from <10 to 1100 μg/L (Durum *et al.* 1971). Although major sources of arsenic in the environment are the smelting of nonferrous ores, primarily copper, lead, and zinc, and certain coal-fired power plants using arsenic-rich coal (Fowler 1977), substantial arsenic contamination in water can occur from the improper use of arsenical pesticides (Sandhu 1977).

Considerable amounts of data have been compiled on the effects of arsenic on aquatic organisms, particularly freshwater forms (McKee and Wolf 1963, Becker and Thatcher 1973, U.S. Environmental Protection Agency 1976). Arsenic toxicity may vary depending upon factors such as water quality, the particular compound being tested, and the test species. It appears to be less

0090–4341/80/0009–0053 $02.20
1980 Springer-Verlag New York Inc.

toxic to aquatic organisms than other elements, such as mercury, silver, and cadmium, but has been shown to be toxic to some species within the above reported range for arsenic in U.S. waters (Sanders and Cope 1966, Hughes and Davis 1967, Biesinger and Christensen 1972, Speyer 1974, Speyer and Leduc 1975). Studies by Gilderhus (1966), Woolson (1975), Sorenson (1976), and Sandhu (1977) indicate that arsenic is accumulated by aquatic organisms.

Both inorganic and organic forms of arsenic occur in the environment (Braman 1975). Although the biomethylation of inorganic compounds by microorganisms has long been known, little is known about the comparative toxicity of different arsenic forms to aquatic animals and their accumulation in animal tissues.

The purpose of this study was to determine and compare the toxicity of two inorganic and two organic arsenic compounds to several freshwater invertebrate species and one species of fish and the bioaccumulation of these compounds. Determinations of toxicity and accumulation were based on exposures to two concentrations of each arsenic form.

Materials and Methods

Water Characteristics

Untreated Lake Superior water was heated and used in the test at 14° to 16°C. Chemical characteristics of the test water were determined weekly according to methods described by the American Public Health Association *et al.* (1975). Ranges for these measurements were (in mg/L): dissolved oxygen, 6.2 to 8.9; hardness, 42 to 45 as $CaCO_3$; alkalinity, 40 to 44 as $CaCO_3$; and acidity, 3.2 to 5.7 as $CaCO_3$. The pH ranged from 6.9 to 7.3. The slight variation in the above chemical measurements did not significantly affect the measured arsenic concentrations in this test.

Exposure System

The test was conducted with an intermittent-flow exposure system consisting of a multi-channel toxicant injection system (DeFoe 1975) which delivered the four toxicants at two concentrations (100 and 1000 μg/L, nominal) and a control to duplicate exposure chambers. The test chambers were glass aquaria 30 × 60 × 30 cm, with a water volume of 43 L. Flow rate to each chamber was 500 ml every two min, providing a 95% replacement of the test water every nine hr (Sprague 1969).

Fluorescent bulbs provided a light intensity of 20 to 40 lumens at the water surface. An automatically controlled 16-hr photoperiod was used.

A static system utilizing test water from the flow-through system was also employed for a 3-wk life-cycle test with *Daphnia magna* according to procedures described by Biesinger and Christensen (1972). Solutions of arsenic were renewed and analyzed each week during the test.

Toxicant Solution

Stock solutions were made by dissolving reagent-grade arsenic trioxide (As_2O_3), arsenic pentoxide ($3As_2O_5 \cdot 5H_2O$), sodium dimethyl arsenate [$(CH_3)_2AsO(ONa)$](SDMA), and disodium methyl arsenate [$CH_3AsO(ONa)_2 \cdot 6H_2O$](DSMA) in 1 L of deionized water. Ten grams of sodium hydroxide had to be added to sufficiently enhance the solubility of arsenic trioxide in the 1-L solution. Solutions of arsenic trioxide and arsenic pentoxide are referred to in the text as As III and As V, respectively, since these oxidation states were characteristic of each compound after they were

dissolved in the test water under the present test conditions. Stock solutions were placed in syringes, and amounts were added to the test water to achieve the desired concentration. One-hundred-milliliter samples for water analysis were taken twice a week from the middle of each glass aquarium and were immediately analyzed on an atomic absorption spectrophotometer equipped with a HGA 2000 graphite furnace. The detection limit for this procedure was 6 μg/L. Concentrations were measured as total arsenic and are included with standard deviations in Tables 1 and 2. The mean percentage recovery and standard deviation for this procedure was 102 ± 2.7 for nine spiked samples.

Biological Procedures

The flow-through tests were conducted for 28 days. Species exposed to arsenic were the stonefly *Pteronarcys dorsata*, the snail *Helisoma campanulata*, the snail *Stagnicola emarginata*, the amphipod *Gammarus pseudolimnaeus*, and rainbow trout (*Salmo gairdneri*) parr. Weights of the test animals were (in grams): stoneflies, 0.1 to 0.6; snails, 0.1 to 0.4; amphipods, 0.004 to 0.02; and trout, 9.5 to 21.9. Invertebrates were collected from the Blackhoof River near Carlton, Minnesota, Gooseberry River near Two Harbors, Minnesota, and the Eau Clair River near Gordon, Wisconsin, by using a rake and net. Rainbow trout parr were obtained from the Genoa National Fish Hatchery in Wisconsin.

Static tests with *Daphnia* were conducted for three weeks according to procedures described

Table 1. Percentage survival of stoneflies (*Pteronarcys dorsata*), snails (*Helisoma campanulata* and *Stagnicola emarginata*), and rainbow trout (*Salmo gairdneri*) exposed to two concentrations of four arsenic compounds for 28 days. Values for amphipods (*Gammarus pseudolimnaeus*) are for an exposure of 14 days[a]

Measured concentrations, as arsenic (μg/L)	Stonefly		Snail (*H. campanulata*)		Snail (*S. emarginata*)		Amphipod		Rainbow trout	
	A	B[b]	A	B	A	B	A	B	A	B
Control										
<6	100	90	100	100	90	80	80	100	100	100
<6	100	100	100	100	90	80	100	100	100	100
Arsenic III										
88[c] ± 17	100	100	100	100	100	100	90	80	100	100
961 ± 51	100	100	100	90	100	100	0	0	90	100
Arsenic V										
89 ± 17	100	100	80	100	100	100	90	80	100	90
973 ± 55	100	100	80	100	90	90	80	80	100	100
DSMA										
86 ± 29	100	100	90	100	100	100	80	90	100	100
970 ± 100	100	100	100	100	80	100	100	60	100	100
SDMA										
85 ± 21	100	100	100	100	83	100	90	100	100	90
846 ± 235	90	90	100	68	100	100	80	100	100	100

[a] Measured concentrations of arsenic after 14 days were not significantly different from the 28 day values listed above

[b] Duplicate chamber

[c] Mean and S.D. of 8 samples

Table 2. Survival and reproduction of *Daphnia magna* exposed to two concentrations of four arsenic compounds for 14 days

Measured concentrations, as arsenic (μg/L)	Survival (%)				Young production[a]			
	A	B	C	D[b]	A	B	C	D
Control								
<6.0	100	100	100	100	45	22	16	8
Arsenic III								
94[c] ± 11	100	100	100	100	27	17	29	12
955 ± 79	100	100	100	80	30	32	29	0
Arsenic V								
89 ± 12	100	100	100	100	17	17	5	8
932 ± 84	100	100	100	100	38	59	7	12
DSMA								
88 ± 9	100	100	100	100	78	22	9	10
833 ± 90	100	100	100	100	35	8	15	5
SDMA								
89 ± 8	100	100	100	100	40	3	2	6
1112 ± 299	100	100	100	100	21	7	14	5

[a] Total number of young produced
[b] Replicate chamber
[c] Mean and S.D. of 4 samples

by Biesinger and Christensen (1972). Daphnia were obtained from the culture unit at the Environmental Research Laboratory-Duluth, Duluth, Minnesota.

All animals except *Daphnia* were acclimated in the flow-through test system for one week at the test temperature before the start of the test. Ten animals of each species were used per duplicate chamber. Invertebrate species were placed in round stainless steel screen containers (6 cm diameter × 15 cm deep) that were suspended in the test aquaria. Fish were located in the same test aquaria, but were separated from the invertebrates by a stainless steel screen partition. Invertebrate species were fed fall-dropped birch and aspen leaves presoaked in diluent water for at least one month. Fish were fed commercial trout food pellets, approximately six g per tank per day.

Deaths were recorded after the fourth day and then once a week for the remainder of the test. Death was defined as complete immobilization and failure of the animal to respond to prodding.

Residue Analysis

After 14 and 28 days of exposure, test animals were killed, rinsed in control water, and frozen for residue analysis. Five whole individuals exposed to 1000 μg/L of each compound and two individuals exposed to 100 μg/L of each compound were analyzed. Amphipods and daphnids were pooled as a group sample per compound because of their small size (<0.02 g, dry weight). All animals were digested by using a method described by Leonard (1971). After digestion, residues were assayed on a dry-weight basis by atomic absorption spectrophotometry employing a graphite furnace. The detection limit for this procedure was 1.0 μg/g for all animals except amphipods and daphnids where the limit was higher (5.0 μg/g) because of the small sample size. The mean recovery and standard deviation for this method was 100% ± 7.0% for nine spiked samples. The results of analysis of two orchard leaf samples from the National Bureau of Standards were 12.3 and 16.2 μg/g, compared to a measured value of 14 ± 2 μg/g from this procedure.

Bioconcentration factors (BCF) were calculated by dividing the residue tissue concentration by the corresponding exposure concentration.

Statistical Analysis

Percentage survival of the test organisms was determined after 14 days of exposure based on the initial number of animals and again after the second 14-day period based on the number of animals remaining (usually five per duplicate chamber) after samples were taken for residue analysis. Total percentage survival after 28 days was calculated by multiplying the two percentages. Both survival and accumulation data were subjected to one-way analysis of variance ($P = 0.05$) and to Duncan's multiple range test for comparisons among treatment means ($P = 0.05$) (Steel and Torrie 1960).

Results

Survival

None of the arsenic compounds caused significant decreases in the survival of stoneflies, snails, or trout after 28 days of exposure (Table 1). However, arsenic III (961 μg As/L) significantly decreased the survival of amphipods to 20% after 7 days and killed all of the animals after 14 days. Although percentage survival was as low as 20 to 40% in exposure of amphipods to the other arsenic compounds after 28 days, significant statistical differences ($P = 0.05$) were not determined for this period and the data was not tabulated due to the large variability in response between replicate chambers. The variability in percentage survival of amphipods, especially in one control chamber after this period, is not clearly understood. Factors such as an inappropriate food supply or the use of organisms of this species which were near the end of their life cycle could have accounted for this discrepancy. This result was also complicated by the use of a small number of organisms, usually five per replicate chamber after thinning for residue analysis (after 14 days), which accounted for low survival with only one or two deaths.

Accumulation

Arsenic accumulation in stoneflies and snails exposed to 1000 μg As/L of the four arsenic compounds was significantly higher than that in the control animals after 14 and 28 days (Figures 1, 2, and 3). Stoneflies accumulated similar residue concentrations when exposed to the high concentration of each of the compounds after 28 days (Figure 1). Mean residue concentrations ranged from 29 ± 16 to 44 ± 15 μg/g. Bioconcentration factors for this concentration ranged from 33 to 45. Stoneflies at the low arsenic concentration accumulated the highest residues (12 ± 5 μg/g) when exposed to arsenic V, which resulted in a BCF of 131. The snail *H. campanulata* accumulated the highest arsenic residues (80 ± 64 μg/g) when exposed to arsenic III (961 μg As/L) (Figure 2) and resulted in a BCF of 83. This snail, however, had a slightly greater BCF of 99 with a residue content of 8.8 ± 1.6 μg/g when exposed to arsenic V (89 μg As/L). The snail *S. emarginata* accumulated similar arsenic residues when

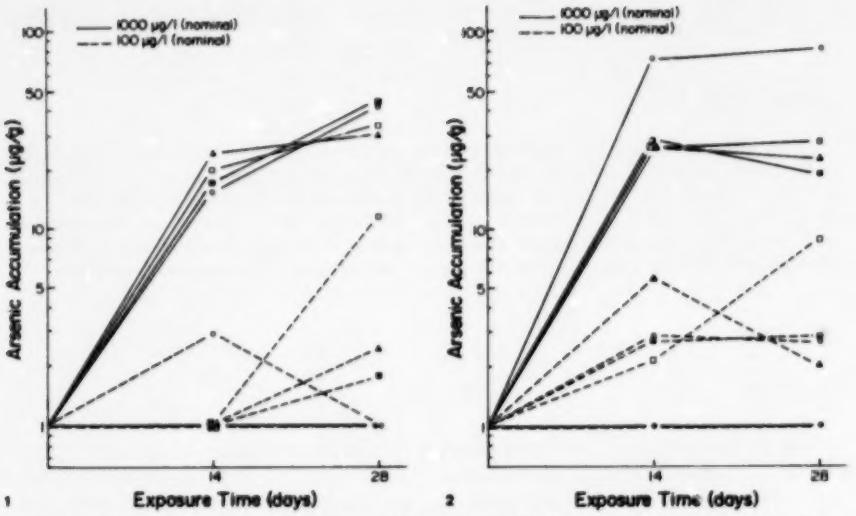

Fig. 1. Total arsenic accumulation (dry weight) in stoneflies (*P. dorsata*) exposed to two concentrations of arsenic III (○), DSMA (■), arsenic V (□), and SDMA (▲) for 28 days. Each symbol represents the mean of 5 samples in the solid lines and 2 samples in the dashed lines. ● indicates control value

Fig. 2. Total arsenic accumulation (dry weight) in the snail *H. campanulata* exposed to two concentrations of arsenic III (○), DSMA (■), arsenic V (□), and SDMA (▲) for 28 days. Each symbol represents the mean of 5 samples in the solid lines and 2 samples in the dashed lines. ● indicates control value

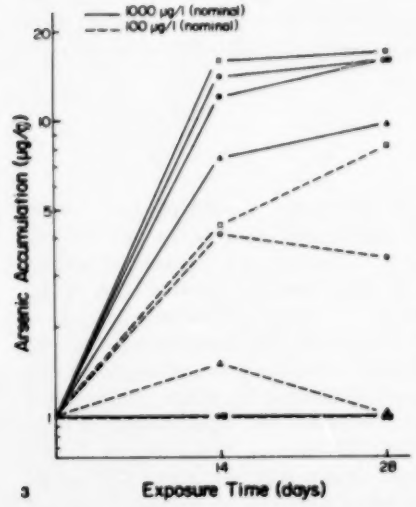

Fig. 3. Total arsenic accumulation (dry weight) in the snail *S. emarginata* exposed to two concentrations of arsenic II (○), DSMA (■), arsenic V (□), and SDMA (▲) for 28 days. Each symbol represents the mean of 5 samples in the solid lines and 2 samples in the dashed lines. ● indicates control value

exposed to the high concentration of arsenic III, arsenic V, and DSMA (Figure 3). The BCF's for this concentration ranged from 16 to 17. Residue concentrations were significantly less in animals exposed to SDMA. As with *H. campanulata* and stoneflies, residues in *S. emarginata* exposed to 89 μg As/L were highest (8.2 ± 0.5 μg/g) when the animals were exposed to arsenic V, resulting in a BCF of 92.

Amphipods did not accumulate arsenic above the detection limit of 5 μg/g when exposed to any of the compounds after 28 days. Arsenic residues in treated rainbow trout were generally the same as those in control fish (approximately 3.0 μg/g) after this period. A significantly higher residue concentration of 4.6 ± 0.1 μg/g in trout exposed to DSMA (86 μg As/L) may have been due to analytical variation since all values were close to the detection limit for this procedure.

Daphnia Chronic Test

None of the four arsenic compounds significantly reduced survival or young production of *Daphnia magna* after 14 days of exposure (Table 2). Percentage survival and young production was not tabulated for *Daphnia* exposed for 21 days, since a number of unexplained deaths occurred in two of the four replicate control chambers during the last few days of the test and significant statistical differences could not be shown.

Arsenic accumulation in *Daphnia magna* increased with increased exposure concentration, and residues were highest in daphnids exposed to arsenic III (Figure 4). Bioconcentration factors calculated for animals exposed to arsenic III (970 and 96 μg As/L) were 50 and 219, respectively after 21 days. Residues in daphnids exposed to the low concentration of the other compounds were similar to the controls after this period.

Discussion

A concentration of 1000 μg As/L as arsenic III was lethal to amphipods within one week, but the same concentration of arsenic supplied as arsenic V, DSMA, and SDMA did not significantly decrease the survival of amphipods and *Daphnia* after two weeks of exposure and other species including stoneflies, snails, and rainbow trout after 28 days. The greater toxicity of arsenic III relative to the other arsenic compounds in this test corroborates the report by Ferguson and Gavis (1972) of greater toxicity in lower oxidation state arsenicals (+3) than in the more oxidized (+5) forms. The mechanism of toxicity of arsenic is due to the reaction of arsenic III with sulfhydryl groups of proteins causing enzyme inactivation (Webb 1966, Peoples 1975). Pentavalent arsenicals do not appear to react with sulfhydryl groups. The toxicity of methylarsenicals and most organic arsenicals to aquatic organisms has not been evaluated. Although the organic compounds in this test did not appear to be toxic, other studies determining their toxicity are needed, since organic forms are readily transformed

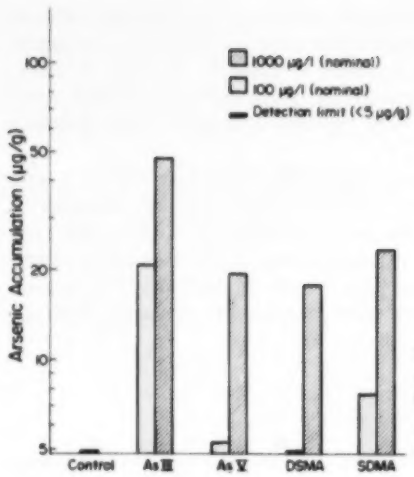

Fig. 4. Total arsenic accumulation (dry weight) in *Daphnia magna* exposed to two concentrations of four arsenic compounds for 21 days. Analysis was made from pooled samples (see text)

from inorganic arsenic in water, microorganisms, and fish (Ferguson and Gavis 1972, Braman 1975, Penrose 1975, Irgrolic *et al.* 1977).

In this study, amphipods were the most sensitive species to arsenic. Surber and Meehean (1931) similarly found amphipods (*Hyalella knickerbocker*) to be among the most sensitive organisms to arsenic III, whereas damselflies, dragonflies, and isopods were much more tolerant, surviving concentrations of 10,500 to 21,000 µg/L. Stoneflies (*Pteronarcys californica*) appear to be highly tolerant (96-hr LC50 of 38,000 µg/L) to arsenic III (Sanders and Cope 1968).

Rainbow trout (Hale 1977) and other fish species (Inglis and Davis 1972, Cardwell *et al.* 1976, Sorenson 1976) had extremely high acute values (96-hr LC50 of 10,800 to 100,000 µg/L) indicating that fish are relatively tolerant to arsenic. However, little work has been done on long-term effects of arsenic at sublethal concentrations. Speyer (1974) and Speyer and Leduc (1975) found that 1000 µg As/L decreased growth (reduced fat weight gain) of juvenile rainbow trout after 21 days of exposure. Gilderhus (1966) reported that applications of 4000 µg/L sodium arsenite (2,320 µg/L as arsenic) reduced survival and growth of bluegills in outdoor pools after 16 weeks. Immature fish were more sensitive than adults. This concentration also caused pathological changes in some adult fish. Additional long-term studies or studies involving sensitive life stages such as embryos, larvae, or early juveniles (McKim 1977) would more accurately assess the toxicity of arsenic to fish.

In our tests, stoneflies, snails, and daphnids accumulated greater amounts of arsenic (up to 219 times the water concentration) than fish after 28 days of exposure. The lack of arsenic accumulation in fish has been reported in other studies with fish exposed to low arsenic concentrations (Ullman *et al.* 1961, Isensee *et al.* 1973). Isensee *et al.* (1973) reported that lower link organisms such as algae and daphnids had higher BCF's than higher link organisms such as fish in a model ecosystem study, which indicates that arsenic does not

biomagnify up the food chain. Woolson (1975), in a review of the bioaccumulation of arsenicals, similarly reported that arsenic is bioconcentrated by aquatic organisms but not biomagnified. The reason for the low toxicity and low accumulation of arsenic in higher forms such as fish is not clearly known, but fish may have the ability to metabolize arsenic more efficiently than lower food-chain organisms. Sorenson (1976) found that green sunfish apparently were able to remove arsenic from the body after short periods of time. Another possibility is that smaller invertebrate organisms have higher ratios of surface area to mass than fish and therefore can absorb more arsenic after exposure (Isensee *et al.* 1973).

Arsenic accumulation in stoneflies and snails was higher when animals were exposed to higher exposure concentrations and appeared to reach a steady state after 14 days. This pattern of uptake is similar to that in aquatic animals exposed to heavy metals such as cadmium and zinc (Benoit *et al.* 1976, Spehar 1976, Spehar *et al.* 1978).

Total arsenic accumulation in stoneflies and snails exposed to 1000 μg As/L did not appear to be greatly affected by the form of arsenic in the water, although some animals exposed to the inorganic forms had higher residues. All animals exposed to the lower concentration (100 μg As/L) accumulated the greatest amounts of arsenic when exposed to inorganic arsenic, particularly arsenic V. It is not evident from this study why greater arsenic accumulation occurred during exposure to inorganic arsenic; however, reduced residues in animals exposed to organic forms were probably the result of the unavailability of arsenic in the water after reduction and subsequent volatilization. Loss rates of arsenic appear to increase slightly with increased methylation of the compound (Woolson 1977).

Arsenic does not appear to accumulate in some aquatic species to as high a degree as other elements. Concentrations of arsenic accumulated in stoneflies and snails in this test were up to 130 times the concentration in the water, whereas the same and similar species contained up to 30,000 and 9,000 times the amount of cadmium and lead, respectively, in the water after the same period of time (Spehar *et al.* 1978). Studies by Spehar (1976), Benoit *et al.* (1976), and Holcombe *et al.* (1976) showed that fish accumulate significant amounts of cadmium and lead after exposure to these metals compared to the low accumulation of arsenic by fish in our study. The lower accumulation of arsenicals than metals in aquatic organisms is probably due to the complex transformations of arsenic (Woolson 1975) resulting in loss of the compound by reduction to highly volatilized forms. Penrose (1975) and Irgrolic *et al.* (1977) indicate that species such as brown trout and *Daphnia magna* are capable of converting inorganic arsenic to some type of organic form. Research concerning the reactions and identification of arsenical compounds in aquatic organisms would aid significantly in our understanding of the toxicity and accumulation of arsenic and possibly other similar toxicants.

In summary, inorganic arsenic appears to be more toxic and slightly more accumulated in some aquatic species than organic arsenic. Inorganic forms such as arsenic III may have adverse effects on aquatic organisms at concentrations within the range reported for arsenic in some lakes and rivers. Arsenic bioconcentrated particularly in lower level food-chain organisms, but not to as high a degree as some metal forms.

References

American Public Health Association, American Water Works Association and Water Pollution Control Federation: Standard methods for the examination of water and wastewater. 14th ed., Washington, DC (1975).

Becker, C. D., and T. O. Thatcher: Arsenates and arsenites. pp. C1-C11. In Toxicity of power plant chemicals to aquatic life. U.S. Atomic Energy Commission WASH 1249. Richland, WA, Batelle Memorial Institute, Pacific Northwest Laboratories (1973).

Benoit, D. A., F. N. Leonard, G. M. Christensen, and J. T. Fiandt: Toxic effects of cadmium on three generations of brook trout (*Salvelinus fontinalis*). Trans. Am. Fish. Soc. **105**, 550 (1976).

Biesinger, K. E., and G. M. Christensen: Effects of various metals on survival, growth, reproduction, and metabolism of *Daphnia magna*. J. Fish. Res. Board Can. **29**, 1691 (1972).

Braman, R. S.: Arsenic in the environment. In E. A. Woolson (ed.): Arsenical pesticides (ACS Symp. Ser. 7). Washington, DC: American Chemical Society (1975).

Cardwell, R. D., D. G. Foreman, T. R. Payne, and D. J. Wilbur: Acute toxicity of selected toxicants to six species of fish. Ecol. Res. Series EPA-600/3-76-008. U.S. Environ. Prot. Agency, 125 p. (1976).

DeFoe, D. L.: Multichannel toxicant injection system for flow-through bioassays. J. Fish Res. Board Can. **32**, 544 (1975).

Durum, W. H., J. D. Hem, and S. G. Heidel: Geo. Survey Circ. 643 (1971).

Ferguson, J. F., and J. Gavis: A review of the arsenical cycle in natural waters. Water Res. **6**, 1259 (1972).

Fowler, B. A.: International conference on environmental arsenic: An overview. Environ. Health Perspectives **19**, 239 (1977).

Gilderhus, P. A.: Some effects of sublethal concentrations of sodium arsenite on bluegills and the aquatic environment. Trans. Am. Fish. Soc. **95**, 289 (1966).

Hale, J. G.: Toxicity of metal mining wastes. Bull. Environ. Contam. Toxicol. **17**, 66 (1977).

Holcombe, G. W., D. A. Benoit, E. N. Leonard, and J. M. McKim: Long-term effects of lead exposure on three generations of brook trout (*Salvelinus fontinalis*). J. Fish Res. Board Can. **33**, 1731 (1976).

Hughes, J. S., and J. T. Davis: Effects of selected herbicides on bluegill sunfish. In Proc. 18th Ann. Conf., S.E. Assoc. Game and Fish Comm. October 18-21. Columbia, SC, S.E. Assoc. Game Fish Comm. (1967).

Inglis, A., and E. L. Davis: Effects of water hardness on the toxicity of several organic and inorganic herbicides to fish. Bur. Sport Fish Wildl. Tech. Paper 67. U.S. Dept. Interior (1972).

Irgrolic, K. J., E. A. Woolson, R. A. Stockton, R. D. Newman, N. R. Bottino, R. A. Zingaro, P. C. Kearney, R. A. Pyles, S. Maeda, W. J. McShane, and E. R. Cox: Characterization of arsenic compounds formed by *Daphnia magna* and *Tetraselmis chuii* from inorganic arsenate. Environ. Health Perspectives **19**, 61 (1977).

Isensee, A. R., P. C. Kearney, E. A. Woolson, G. E. Jones, and U. P. Williams: Distribution of alkyl arsenicals in model ecosystems. Environ. Sci. Tech. **7**, 841 (1973).

Leonard, E. N.: The determination of copper in fish tissues by atomic absorption spectrophotometry. Atomic Absorp. Newsl. **10**, 84 (1971).

McKee, J. E., and H. W. Wolf: Water quality criteria. The Resources Agency of California. State Water Quality Control Board Publ. No. 3-A. Sacramento, CA (1963).

McKim, J. M.: Evaluation of tests with early life stages of fish for predicting long-term toxicity. J. Fish. Res. Board Can. **34**, 1148 (1977).

Penrose, W. R.: Biosynthesis of organic arsenic compounds in brown trout (*Salmo trutta*). J. Fish. Res. Board Can. **32**, 2385 (1975).

Peoples, S. A.: Review of arsenical pesticides. In E. A. Woolson (ed.): Arsenical pesticides (ACS Symp. Ser. 7) Washington, DC: American Chemical Society (1975).

Sanders, H. O., and O. B. Cope: Toxicities of several pesticides to two species of cladocerans. Trans. Am. Fish. Soc. **95**, 165 (1966).

———— ———— —— ———— ————: The relative toxicities of several pesticides to naiads of three species of stoneflies. Limnol. Oceanogr. **13**, 112 (1968).

Sandhu, S. S.: Study on the post-mortem identification of pollutants in the fish killed by water pollution: Detection of arsenic. Bull. Environ. Contam. Toxicol. **17**, 373 (1977).

Sorenson, E. M. B.: Toxicity and accumulation of arsenic in green sunfish, *Lepomis cyanellus* exposed to arsenate in water. Bull. Environ. Contam. Toxicol. **15**, 756 (1976).

Spehar, R. L.: Cadmium and zinc toxicity to flagfish, *Jordanella floridae*. J. Fish. Res. Board Can. **33**, 1939 (1976).

Spehar, R. L., R. L. Anderson, and J. T. Fiandt: Toxicity and bioaccumulation of cadmium and lead in aquatic invertebrates. Environ. Pollut. **15**, 195 (1978).

Speyer, M. R.: Some effects of combined chronic arsenic and cyanide poisoning on the physiology of rainbow trout. M.S. Thesis, Concordia Univ., Montreal, Canada (1974).

Speyer, M. R., and G. Leduc: Effects of arsenic trioxide on growth of rainbow trout. In International conference on heavy metals in the environment. Toronto, Ontario, Canada October (1975).

Sprague, J. B.: Measurements of pollutant toxicity to fish. 1. Bioassay methods for acute toxicity. Water Res. **3**, 793 (1969).

Steel, R. G. D., and J. H. Torrie: Principles and procedures of statistics with special reference to the biological sciences. New York: McGraw Hill (1960).

Surber, E. W., and O. L. Meehean: Lethal concentrations of arsenic for certain aquatic organisms. Trans. Am. Fish. Soc. **61**, 225 (1931).

Ullman, W. W., R. W. Schaefer, and W. W. Sanderson: Arsenic accumulation by fish in lakes treated with sodium arsenite. J. Water Poll. Control Fed. **33**, 416 (1961).

U.S. Environmental Protection Agency: Arsenic. Subcommittee on arsenic. Committee on medical and biological effects of environmental pollutants, National Research Council, National Academy of Sciences. EPA-600/1-76-036. Office of Research and Development, Health Effects Research Laboratory, Research Triangle Park, NC (1976).

Webb, J. L.: Enzyme and metabolic inhibitors. Vol. III, Chapter 6. New York: Academic Press (1966).

Woolson, E. A.: Bioaccumulation of arsenicals. In E. A. Woolson (ed.): Arsenical pesticides (ACS Symp. Ser. 7). Washington, DC: American Chemical Society (1975).

———— ————: Fate of arsenicals in different environmental substrates. Environ. Health Perspectives **19**, 73 (1977).

Woolson, E. A., A. R. Isensee, and P. C. Kearney: Distribution and isolation of radioactivity from ^{74}As-arsenate and ^{14}C-methanearsonic acid in an aquatic model ecosystem. Pestic. Biochem. Physiol. **6**, 261 (1976).

Manuscript received February 16, 1979; accepted May 18, 1979.